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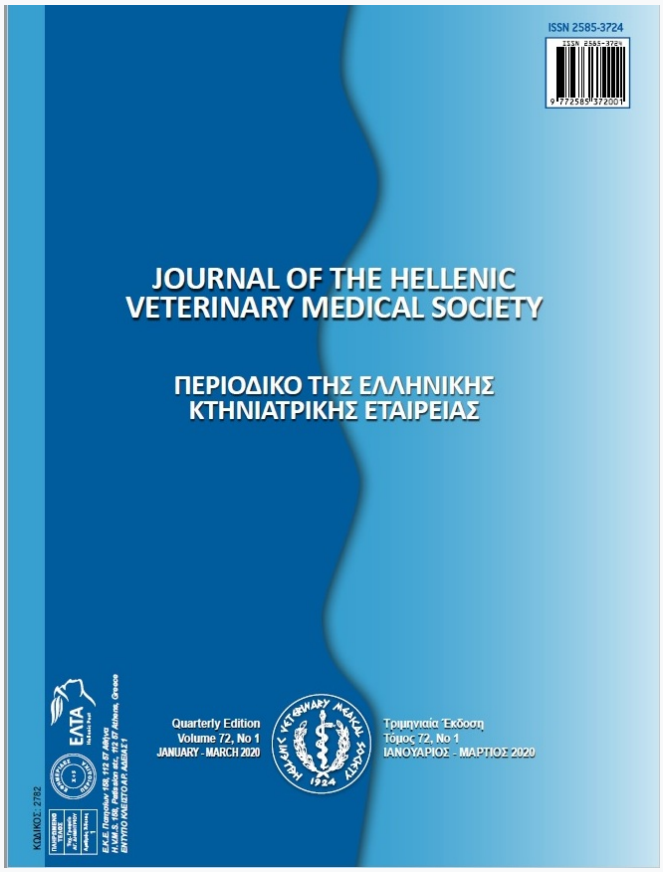

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Assessment of the Microbiological Profile, Species Diversity and Antimicrobial Susceptibility of Recovered Bacteria from Retail Honeys in Turkey

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ABSTRACT: The aim of this study was to assess the microbiological profile, coliform and staphylococcal species diversity, and the antimicrobial susceptibility of coliform and other Gram-negative bacteria recovered from retail honeys in Turkey. A total of 150 honey samples, including extracted honey and comb honey samples, were purchased from honey sellers. The honey samples were analyzed for total mesophilic aerobic bacteria (TMAB), total mesophilic anaerobic bacteria (TMAnB), coliforms, *Escherichia coli* (*E. coli*), *Staphylococcus* spp., lactic acid bacteria (LAB), yeasts, and molds. All presumptive coliform and Staphylococcus isolates were identified at species level and then Gram-negative isolates were screened for antimicrobial susceptibility. TMAB, TMAnB, LAB, yeasts and molds mean counts (log cfu/g) in the samples were 3.26±1.08, 3.0±0.89, 2.93±0.52, 2.90±0.83, 1.80±0.53, respectively. Eighteen point seven percent and 15.3% of extracted and comb honey contained coliform and *Staphylococcus* spp., respectively, with a mean count (MPN/g) of 8.06±1.23 and 0.71±0.66. TMAB, *Staphylococcus* spp. and yeast contamination rates were significantly higher in the extracted honeys ($P<0.05$). Presumptive coliform and *Staphyloccus* spp. isolates were mostly identified as *Klebsiella pneumoniae, Proteus mirabilis, Serratia marcescens,* and *Staphylococcus hominis* and *Staphylococcus epidermidis*, respectively. Among coliform and non-coliform Gram-negative recovered isolates, antimicrobial resistance was highest against ceftriaxone (92.4%) and cefepime (91.5%) followed by tigecyclin (46.2%). The results obtained in this study provide insight on the microbiological profile of honey and the diversity of coliform and Staphylococcus species in honey samples. Moreover, these results show that honey, which is considered beneficial for human health, may contain antibiotic-resistant bacteria.

***Keywords*:** Honey, microbiological profile, coliform, Staphylococcus, antimicrobial susceptibility

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INTRODUCTION

Owing to its several beneficial biological effects, including antioxidant (Aljadi and Kamaruddin, 2004), antimicrobial (Gomes et al., 2010), and anti-inflammatory (Tonks et al., 2003) activity, the consumption of honey, produced by honeybees (*Apis mellifera*), contributes to the health and well-being of humans. In the past few years, the production and consumption of honey have displayed a steady increase at the global level. This is attributed to the increase in the global population, the interest of consumers of all age groups, including young people, in natural food products, and the variety of food products containing honey (Garcia, 2018). To date, literature reports on honey have mostly focused on the physicochemical properties (El Sohaimy et al., 2015; Chakir et al., 2016; Boussaid et al., 2018; Kavanagh et al., 2019) and antimicrobial activity (Mercan et al., 2007; Sherlock et al., 2010; Stagos et al., 2018) of honey. However, although honey stops the growth of many microorganisms because of its composition (high concentration of sugar and a low water activity), throughout the different stages of the production chain from the hive to the table [primary (pollen, flower, honeybee digestive tract) and secondary (human, equipment, containers, wind, dust, soil etc.)], honey can be contaminated by microorganisms, which may alter the shelf life of the product and/or cause foodborne diseases (Olaitan et al., 2007; Grabowski et al., 2017). Honey being consumed without undergoing any prior heat treatment or preservation techniques requires strict attention to be paid to good manufacturing practices during its production. Previously reported studies from different countries have shown that retail honey can contain vegetative and spore-forming bacteria, yeast and mold (Ceauşi et al., 2009; Kačániová et al., 2012; Dümen et al., 2013; Erkan et al., 2015; Kunová et al., 2015; Moujanni et al., 2017; Combarros-Fuertes et al., 2019). Besides, most of these studies focused on determining microbiological profile rather than microbial diversity at species level from the samples. Mostly, the reported studies for microbiological profile varies globally but these are influenced by the detection methods which have different sensitivity and specificity, the region and the study design. In recent years, the rapid development of antibiotic resistance in several bacteria, and reports showing the role of bacteria originating from food, animals, and the environment in certain infectious diseases affecting humans (Manges, 2016; Bhatta et al., 2016; WHO, 2016), have increased the importance of genus/species identification and antimicrobial resistance detection in bacteria isolated not only from human clinical specimens but also from food, animal and environmental samples. Antibiotic resistant Gram negative bacteria are a serious problem in clinical settings and increase the morbidity and mortality in humans (Cosgrove, 2006; Kollef et al., 2008). There is very limited data in literature on antibiotic resistant Gram negative bacteria, including coliforms, isolated from honey (Hleba et al., 2014). However, the few reported studies on isolates from the digestive tracts of honey bees have found the Gram negative bacteria to be resistant to different antibiotic classes (Tian et al., 2012; Bezirtzoglou et al., 2016; Gasper et al., 2017; Kačániová et al., 2017). To the best our knowledge, there is no previous study that systemically focused on the determination of microbiological profile including coliform and Staphylococcus species diversity and antibiotic susceptibility in coliform and Gram negative bacteria recovered from retail honeys in Turkey. Therefore, the aim of this study was i) to assess the microbiological profile of honeys ii) to determine both coliform and Staphylococcus isolates at genus and species level, and iii) to screen antimicrobial susceptibility in the recovered coliform and non-coliform Gram negative bacteria isolates.

MATERIAL AND METHODS

Study design and sample collection

A cross-sectional study was conducted from July 2017 to June 2018 in the Diyarbakir province located in the Southeast Anatolia Region of Turkey. In total, 150 honey samples were collected from different sale points including honey sellers and markets in four districts (Baglar, Kayapinar, Yenisehir and Sur) of Diyarbakir province. The number of samples per area was determined according to the relative population size of the districts (TUİK, 2016). The sample numbers of the analyzed honey types (extracted or comb honey) were determined in view of the consumption levels of extracted honey and comb honey in Turkey (Soylu et al., 2018; Baki et al., 2017). Eventually, 106 extracted honey (71%) and 44 comb honey (29%) samples were collected. The samples were collected into sterile 100-ml containers (labelled with numbers, place and date of collection) and transferred in cold boxes at 4°C to the laboratory of the Department of Food Hygiene and Technology of Dicle University for microbiological analysis.

Microbiological analysis

For microbiological analysis, honey samples were

taken aseptically, using a sterile spatula and/or scalpel (for comb honey), from the sample containers. Ten grams of each honey sample was mixed with a ninefold volume of 0.1% peptone water in a sterile plastic bag, and homogenized for 60 s with a stomacher (Easy Mix-G560E, France). Subsequently, 10-fold serial dilutions were prepared of each sample with 0.1% peptone water. The pour plate technique was used for enumerating total aerobic mesophilic bacteria (TMAB), total anaerobic mesophilic bacteria (TMAnB), lactic acid bacteria (LAB), yeasts and molds in the honey samples. The TMAB count was enumerated on plate count agar (PCA) after incubation at 30°C for 72 hours as described in the ISO 4833-1:2013 standard (ISO 2013). The TMAnB count was enumerated on PCA after incubation at 30°C for 72 hours under anaerobic conditions. Lactic acid bacteria were enumerated on de Man Rogosa and Sharpe Agar (MRSA) incubated at 37°C for 48 hours. Molds and yeasts were enumerated on potato dextrose agar supplemented with 10% tartaric acid, which was incubated at 22±1°C for 5-7 days.

Coliform and *E. coli* counts were performed using the most probable number (MPN) method as described in the U.S. Food and Drug Administration's Bacteriological Analytical Manual (FDA/BAM 2002). From each sampling bag containing 10 g of honey + 90 ml of 0.1% peptone water, aliquots of 10 ml, 1 ml and 0.1 ml were taken and transferred to tubes containing 10 ml (double-strength), 10 ml (single-strength), and 10 ml (single-strength) of lauryl sulphate tryptose (LST) broth, respectively. All tubes were incubated at 35°C±0.5°C for 24-48 hours. Briefly, after presumptive positives were determined, confirmation tests were performed by transferring a loopful of suspension into brilliant green lactose broth (BGLB). The MPN was calculated and species distribution was determined using the Vitek 2 system, according to manufacturer's instructions (Biomerieux, France). For *E. coli* counts, a loopful of suspension, from each presumptive positive tube mentioned above, was transferred to a tube containing *E. coli* broth (EC). The EC tubes were incubated at 44.5°C for 24-48±2h and then examined for gas production. After gently agitating each gassing EC tube, a loopful of broth was streaked on Levine's eosin-methylene blue (L-EMB) agar for isolation. The plates were incubated at 35°C±0.5°C for 18-24 h. Then presumptive colonies were confirmed with the Vitek 2 system (Biomerieux, France).

Staphylococcus spp. counts were determined using 9 test tubes containing 10, 9.9, and 9 ml of tryptic soy broth, 10% NaCl, and 1% sodium pyruvate (TSBNS) (three tubes each) as described in the FDA's BAM (FDA/BAM 2001). From each sampling bag containing 10 g of honey + 90 ml of 0.1% peptone water (corresponding to a dilution of 1:10), aliquots of 10 ml, 1 ml and 0.1 ml were taken and transferred to the tubes containing 10 ml of TSBNS broths, respectively. These tubes were incubated at 37°C for 48±2 hours. Briefly, positive tubes were confirmed by streaking a loopful of suspension onto Baird-Parker agar (BPA) and one or more suspected black colonies from each positive BPA plate were confirmed and identified at the species level using the Vitek 2 system (Biomerieux, France). Following confirmation, the MPN of *Staphylococcus* spp. was calculated based on the proportion of confirmed turbid TSBNS tubes for three consecutive dilutions.

Antibiotic susceptibility testing of the isolates

Antibiotic susceptibility tests were performed on all of the coliform and non-coliform isolates obtained from the honey samples. The susceptibility tests were conducted using the BD Phoenix™ 100 Automatic Microbiology Identification System in accordance with the manufacturer's instructions (BD Diagnostic Instrument Systems, Sparks, MD, USA). A Phoenix NMIC-400/ID Panel, of which the following antibiotics were part of, was used: amikacin, amoxicillin-clavulanate, ampicillin, ciprofloxacin, colistin, gentamicin, netilmicin, tigecycline, trimethoprim-sulfamethoxazole, aztreonam, cefepime, ceftazidime, ceftriaxone, imipenem and meropenem. The minimal inhibitory concentration (MIC) values were interpreted as susceptible, intermediate, or resistant according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2017). All isolates with intermediate susceptibility were re-classified as susceptible.

Statistical analysis

Data analysis was performed with the SPSS statistical software version 24 (IBM SPSS, IBM Corporation, USA). The chi-square test was used to compare differences between microorganism presence and the honey types. Statistical differences between the honey types and mean values of microorganisms were determined using Student's t-test. $P < 0.05$ was considered significant.

RESULTS

Microbiological profile in honeys

The mean numbers determined in the 150 honey samples are given Table 1. The mean TMAB, TMAnB, mold and LAB counts (log cfu/g) of the extracted

honey samples, which were 3.37±1.07, 3.07±0.91, 2.94±0.43, and 2.88±0.49, respectively, were higher than the counts of the comb honey samples (Table 1). However, there were no significant differences in the mean counts of microorganisms between the extracted and comb honey samples (P>0.05). Out of the 150 analyzed honey samples, 94 (62.7%), 34 (22.7%), 28 (18.7%), 25 (16.7%), 23 (15.3%), 21 (14%) and 11 (7.3%) were contaminated at detectable levels (>10 cfu/g or >3 MPN/g) of TMAB, TMAnB, coliforms, yeasts, *Staphylococcus* spp., LAB and molds, respectively. None of the samples were contaminated with *E. coli* within a detectable number (<3 MPN/g). Comparison of honey types showed that 49.3%, 14.7% and 14% of the extracted honey samples, and 13.3%, 2% and 1.3% of the comb honey samples were contaminated with TMAB, yeasts and *Staphylococcus* spp., and these contamination rates were found to be statistically significant (P<0.05) (Table 2).

Distribution of coliform and Staphylococcus isolates

Out of the 150 honey samples analyzed in the present study, 28 (18.7%) contained coliforms, resulting in 106 isolates. Forty-three (40.5%) isolates were identified as *K. pnuemonia*, *E. cloacae* and *K. oxytoca*, all of which are coliform bacteria, whilst the remaining 63 isolates (59.4%) were identified as *P. vulgaris*, *S. marcescens* and *P. mirabilis* (Table 3). The analysis of the 150 honey samples for *E. coli* with the most probable number method revealed turbidity in the EC broth tubes of 11 (7.3%) samples. However, none of the suspected isolates obtained from the EC broth tubes were confirmed as *E. coli*. Of the 150 honey samples tested, 23 (15.3%) were found to be contaminated with Staphylococcus, and a total of 30 strains were isolated. Out of the 25 *Staphylococcus* spp. isolates obtained from extracted honey samples, 10 were *S. hominis*, 8 were *S. epidermidis*, 6 were *S. hemolyticus*, and 6 were *S. capitis*. Furthermore, out of the 5 *Staphylococcus* spp. isolates obtained from comb honey samples, 2 were *S. hominis*, 1 was *S. epidermidis*, 1 was *S. hemolyticus*, and 1 was *S. capitis* (Table 3). None of the analyzed honey samples was contaminated with *S. aureus* in detectable numbers.

Table 1. Microbial counts in honeys

Variable	Overall counts (N:150)			Honey types					
				Extracted honey (n:106)			Comb honey (n:44)		
	Range (Min.-Max.)	Mean±SD	Median	Range (Min.-Max.)	Mean±SD*	Median	Range (Min.-Max.)	Mean±SD*	Median
TMAB[x]	1.13-5.08	3.26±1.08	3.41	1.13-5.08	3.37±1.07	3.45	1.17-4.33	2.84±1.04	3.15
TMAnB[x]	1.30-4.37	3.00±0.89	3.04	1.30-4.37	3.07±0.91	3.16	1.77-3.14	2.48±0.55	2.66
Molds[x]	1.17-2.79	1.80±0.53	2.75	1.77-4.39	2.94±0.43	1.78	1.77-3.50	2.64±0.75	2.31
Yeasts[x]	1.77-4.39	2.90±0.83	1.97	1.17-2.10	1.66±0.84	2.75	1.47-2.79	2.19±0.66	2.65
Lactic acid bacteria[x]	1.84-3.99	2.93±0.52	2.90	1.84-3.58	2.88±0.49	2.85	2.70-3.99	3.34±0.91	3.05
Coliform[y]	0.30-46	8.06±1.23	0.74	0.30-29.0	6.76±9.65	0.74	0.30-46.0	12.81±1.98	0.64
Staphylococcus spp.[y]	0.36-2.30	0.71±0.66	0.36	0.36-2.30	0.65±0.59	0.53	0.36-2.30	1.33±1.37	1.33

[x] log cfu/g
[y] MPN/g
SD: Standard deviation
*There was no significant difference in the mean counts of microorganisms between honey types.
None of samples was found to be contaminated with *E. coli* in detectable numbers (<3 log MPN/g).

Table 2. Microbial contamination rates in honey samples (N:150, %)

Microorganism	Honey types		Overall
	Extracted honey (n:106)	Comb honey (n:44)	
TMAB	49.3[a]	13.3[b]	62.7
TMAnB	18.7[a]	4[a]	22.7
Coliform	14.7[a]	4[a]	18.7
Yeasts	14.7[a]	2[b]	16.7
Staphylococcus spp.	14[a]	1.3[b]	15.3
LAB	11.3[a]	2.7[a]	14
Molds	5.3[a]	2[a]	7.3

[a.b.c]: Values in the same row that are not followed by the same uppercase letter are significantly different (P<0.05).

Table 3. Distribution of coliform, non-coliform Gram negative and *Staphylococcus* spp. isolates identified in honeys*

Microorganism		Honey type	
		Extracted honey (no. of isolates)	Comb honey (no. of isolates)
Coliform bacteria	x		
Klebsiella pneumonia	26 (60.4)	23	3
Enterobacter cloacae	11 (25.6)	8	3
Klebsiella oxytoca	6 (14)	6	Not detected
Non-coliform Gram (-) bacteria	y		
Proteus vulgaris	38 (60.3)	24	14
Serratia marcescens	17 (27)	9	8
Proteus mirabilis	8 (12.7)	7	1
***Staphylococcus* spp.**	z		
S. hominis	10 (33.3)	8	2
S. epidermidis	8 (26.6)	7	1
S. hemolyticus	6 (20)	5	1
S. capitis	6 (20)	5	1

* Of the 150 honey samples analyzed, 28 (18.7%) and 23 (15.3%) were contaminated with coliform and Staphylococcus, respectively.
x: No. of isolates with (% of the 43 isolates)
y: No. of isolates with (% of the 63 isolates)
z: No. of isolates with (% of the 30 isolates)

Resistance pattern of the coliform and non-coliform Gram-negative isolates

The antibiotic resistance of the isolates was highest to ceftriaxone (92.4%) and cefepime (91.5%) followed by tigecyclin (46.2%), trimethoprim-sulfamethoxazole (43.4%), netilmicin (43.4%), amoxicillin-clavulanate (43.4%), ceftazidime (36.8 %), aztreonam (36.8%), and colistin (30.1%) (Table 4). The isolates with the highest percentages of resistance to the different antibiotics tested were *P. vulgaris* and *P. mirabilis*. Of the isolates, 83% were found to be multi-drug resistant (resistant to at least three different classes of antibiotics).

Table 4. Antimicrobial susceptibility pattern of coliform and non-coliform Gram negative bacteria recovered from honeys

Bacterial isolate	No. of resistant isolates with (%)										
	AMC	CIP	COL	GEN	NET	TIG	TMP- SUL	AZT	CEF	CEFT	CFTX
***Proteus vulgaris*(n:38)**	38(100)	0(0)	NA	0(0)	38(100)	38(100)	38(100)	0(0)	38(100)	0(0)	38(100)
***Klebsiella pneumonia*(n:26)**	0(0)	0(0)	26(100)	10(38)	0(0)	0(0)	0(0)	26(100)	26(100)	26(100)	26(100)
***Serratia marcescens*(n:17)**	NA	0(0)	NA	0(0)	0(0)	0(0)	0(0)	4(24)	17(100)	4(24)	17(100)
***Enterobacter cloacae*(n: 11)**	NA	3(27)	0(0)	0(0)	0(0)	3(27)	0(0)	3(27)	3(27)	3(27)	4(36)
***Proteus mirabilis*(n:8)**	8(100)	8(100)	NA	0(0)	8(100)	8(100)	8(100)	0(0)	7(87.5)	0(0)	7(87.5)
***Klebsiella oxytoca*(n:6)**	0(0)	0(0)	6(100)	4(67)	0(0)	0(0)	0(0)	6(100)	6(100)	6(100)	6(100)
Overall (n: 106)	46 (43.4)	11(10.3)	32 (30.1)	14 (13.2)	46(43.4)	49(46.2)	46(43.4)	39(36.8)	97(91.5)	39(36.8)	98(92.4)

AMC: Amoxicillin-Clavulanate; CIP: Ciprofloxacin; COL: Colistin; GEN: Gentamicin; NET: Netilmicin; TIG: Tigecycline; TMP-SUL:Trimethoprim-Sulfamethoxazole; AZT: Aztreonam; CEF: Cefepime; CEFT: Ceftazidime; CFTX: Ceftriaxone.
NA: The isolate has intrinsic resistance to certain antibiotic.
All isolates were pan susceptible/sensitive to amikacin, imipenem and meropenem

DISCUSSION

Total mesophilic aerobic bacteria, total anaerobic bacteria, yeast and mold counts are important parameters used to determine the microbial quality of honey. Kunová et al. (2015) reported that the total viable count ranged from 1.87 log cfu/g to 3.87 log cfu/g with a median level of 2.52 log cfu/g, based on the analysis of honey samples originating from the Czech Republic, Slovakia and Germany. Pucciarelli et al. (2014) reported that the mean TMAB count of the analyzed honey in Argentina was as 3.13 log cfu/g, Rozanska and Osek (2012) reported a TMAB count ranging from $1.9x10^2$ cfu/g to $4.6x10^3$ cfu/g for five honey types of different botanical origin in Poland. The results determined in the present study for mean TMAB count is similar with the studies mentioned above. However, in a study on honey samples obtained from different stores in Turkey the determined mean TMAB count was higher at 6.98 log cfu/g (Erkan et al., 2015). The physico-chemical composition of honey is favourable for the survival of the spores and vegetative forms of some anaerobic and facultative bacteria, even if at a low level. In the present study, 22.7% of the 150 honey samples analyzed were contaminated with TMAnB and the mean TMAnB count was 3.0±0.89 log cfu/g. Kačániová et al. (2012) reported vegetative anaerobic bacteria levels of 0% in 20 honey samples from Slovakia and 1% in 20 honey samples from Poland. In the same study, the counts of vegetative anaerobic bacteria in the two positive samples were found to be as 1 log cfu/g and 1.54 log cfu/g. Different from other microorganisms, yeasts and molds are capable of long-term survival and even growth in honey. In the analyzed honey samples, 25 (16.7%) and 11 (7.3%) were contaminated with yeast and mold, respectively, in the present study. Similarly, Moujanni et al. (2017) reported to have detected higher yeasts (40%) than molds (32%) in 109 Moroccan honey samples. These results suggest that honey contamination with yeasts occurs at a higher rate, which is attributed to yeasts having a higher capability of surviving and growing in media with high sugar concentrations (Tysett et al., 1981).

The source of lactic acid bacteria in honey is mainly plants, the digestive tract of honeybees and soil. However, these bacteria, which are widespread in the environment, can pass into honey under improper production, processing and storage conditions. In the present study, the mean number of LAB was determined to be 2.93±0.52 log cfu/g. Similar to the present study, Vazquez-Quiñones (2018) determined the presence of lactic acid bacteria at a level above 2 log cfu/g (>10^2 cfu/g) in honeys from Mexico, and Duman et al. (2008) determined a number of lactic acid bacteria ranging between 10^2 - 10^3 cfu/g in honeys from Turkey.

Coliform bacteria, the counts of which are used as an indicator of the sanitary quality of foodstuffs, belong to four genera, namely, *Escherichia*, *Klebsiella*, *Citrobacter* and *Enterobacter*. Some coliform species has also been known to cause clinically important infections in humans (Armbruster et al., 2017). In two of the very few studies, in which the number of coliform bacteria in honey was determined by the MPN method, Pucciarelli et al. (2014) reported the average number of coliforms as 1.45 MPN/g in honey, whilst Vazquez-Quiñones (2018) reported a coliform number of <3 MPN/g. While these results are quite lower than those obtained in the present study, some other literature reports point out the detection of higher coliform numbers (Combarros-Fuertes et al., 2019; Dümen et al., 2013). The contamination of honey with coliform bacteria may occur via the digestive tract of honeybees, pollens, the environment, equipment and personnel hygiene (Silva et al., 2017). In the present study, none of the honey samples contained a detectable level of *E. coli* by MPN method. Similar to the present study, Leme et al. (2018) and Combarros-Fuertes et al. (2019) reported not to have detected *E. coli* in any of the honey samples they analyzed. On the other hand, Dümen et al. (2013) reported to have detected *E. coli* in 18 (3.6%) out of 500 honey samples, and determined that the number of *E. coli* ranged from <10 cfu/g to $3.4x10^1$ cfu/g. To our knowledge, only very few literature reports are available on the systematic investigation of the species distribution of coliform bacteria contaminating honey. Although honey shows antimicrobial activity against several clinically important pathogens, including *P. mirabilis, P. vulgaris*, *E. cloacae, E. aerogenes* and *K. pneumoniae*, the detection of these bacteria in honey, even at low levels, in the present study, demonstrates that these bacteria can be in honey (Snowdon et al., 1996; McLoone et al., 2016).

Bacteria of the genus Staphylococcus, which are part of the natural microflora of both humans and animals, are ubiquitous and include coagulase-positive and coagulase-negative species, known to bear significance in terms of food safety and public health (Hennekinne et al., 2012; Becker et al., 2014). In a study on honey obtained from beehive combs with sterile syringes it was reported that while 2 (7.14%) of the 28 Yateí honey samples contained coagulase-negative *Staphylococcus* spp., none were contaminated with coagulase-positive *Staphylococcus* spp. (Puciarelli et al., 2014). In another study from different tree spe-

cies, the presence of coagulase-positive *Staphylococcus* spp. was detected in 65 (11.7%) of the 552 honey samples (Ceauşi et al., 2009). When comparing those reports with the present study, the higher contamination rates of *Staphylococcus* spp. in the present study was attributed not only to primary and secondary or cross contaminations, but also to the use of the most probable number method, which enables the detection of *Staphylococcus* spp. numbers less than 10 cfu/g. In the present study, *S. aureus* having not been detected in any of the samples was attributed to the antimicrobial activity of honey against many pathogens, including *S. aureus*, owing to its characteristic composition, structure and microbial flora (Sherlock et al., 2010).

In the present study, it was determined that the overall contamination rate of extracted honey with TMAB, TMAnB, coliforms, *Staphylococcus* spp., yeasts, molds and lactic acid bacteria was higher than that of comb honey. This demonstrated that the microbial quality of extracted honey is relatively lower than that of comb honey. In a study that analyzed honey samples from different points of a honey processing unit was reported that postharvest extracted honey contained increased numbers of TMAB, yeasts and molds, and that coliform bacteria, was not detected in comb honey (Fernandez et al., 2017). In their study on the comparison of the cold and hot extraction methods used to obtain extracted honey from comb honey, Gallez and Fernández (2009) determined that the microbial contamination of extracted honey occurred with the use of both methods and the cold extraction method posed a greater risk of contamination than the hot extraction method. The increased risk of microbial contamination associated with each step of the production of extracted honey from comb honey at honey processing units is in agreement with the lower microbial quality determined for extracted honey, compared to comb honey.

The Turkish Food Codex by-law on microbiological criteria and the 2073/2005 numbered microbiological criteria for foodstuffs of the European Commission (EC) do not enforce any limit for TMAB, TMAnB, coliform, *Staphylococcus* spp., lactic acid bacteria, yeast and mold count of honey (Turkish Food Codex, 2011; European Commission, 2005). Therefore, the microorganism counts determined in the present study were not assessed for conformity to any legal requirements. However, the determined values for the studied microorganisms in the present study are not considered hazardous to humans.

Antibiotic resistance rapidly increasing among bacteria is a major public health concern at the global level (WHO, 2016). The non-legal use of different antibiotic groups, such as tetracyclines, streptomycin, macrolides, and sulphonamides for the treatment of honeybee diseases may cause antibiotic residues in honey. The high levels of multi-drug resistant coliform and non-coliform Gram-negative bacteria detected in the present study not only suggest that drugs are used improperly, but also point out the impact of multiple factors, including the close contact of honeybees with the environment, the vector role of honeybees in transferring antibiotic-resistant bacteria to the hive, the ability of antibiotic-resistant bacteria to colonize in the honeybee gut, and the contamination of honey with antibiotic-resistant bacteria at different stages, from production to the consumer (Tian et al., 2012; Kačániová et al., 2017).

CONCLUSIONS

E. coli and *S. aureus* having not been detected in any of the honey samples analyzed in the present study shows that the sanitary quality of the honey was acceptable. The presence of clinically important bacteria for humans from the analyzed samples indicates that these bacteria can be found in honey. Findings in this study indicated that honey, can act as a potential vehicle for the transmission of antibiotic-resistant bacteria and thus, pose a health risk to consumers.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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